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**Seroepidemiological survey and spatial analysis of the occurrence of
Angiostrongylus vasorum in Swiss dogs in relation to biogeographic aspects**

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Seroepidemiological survey and spatial analysis of the occurrence of
Angiostrongylus vasorum in Swiss dogs in relation to biogeographic aspects

Angiostrongylus vasorum is a nematode living in the pulmonary arteries and in the right heart causing respiratory distress, coagulopathies and other clinical signs in dogs. The aim of this study was to investigate the seroprevalence and distribution of *A. vasorum* in Swiss dogs and to identify correlations with biogeographic aspects. A total of 6136 sera were examined for the presence of circulating parasite antigen and specific antibodies by ELISA. The combined seroprevalence for both specific antibodies and antigen was 0.96% (95% CI: 0.7-1.2%), while the overall antibody prevalence was 3.08% (CI: 2.7-3.5%) and the antigen prevalence 2.17% (CI: 1.8-2.6%). The highest prevalence for dogs identified as positive in both ELISAs was detected in Western Switzerland (around Geneva, 2.21%, CI: 0.7-5.1), representing a new endemic area. Known endemic regions (Ticino, 2.17%, CI: 1.0-4.0 and northern Switzerland, 1.11%, CI: 0.4-2.4) were confirmed. Spatial analysis identified a cluster in Sisseln (AG), for antibody positive dogs. In total 96.6% (57/59) of the antigen- and antibody positive samples originated from areas with a mean temperature warmer than -2°C in January. Correspondingly, 89.8% (53/59) originated from areas below 700 m asl. This study confirms previously known endemic areas and identifies positive dogs in virtually all bioregions. As asymptomatic dogs may suddenly develop potentially fatal symptoms, disease awareness has to be maintained for dogs from across Switzerland at altitudes below 700 m asl.

Angiostrongylus vasorum – dog – antigen detection – antibody detection – spatial analysis

Angiostrongylus vasorum ist ein Nematode, der die rechte Herzkammer und die *Arteria pulmonalis* besiedelt und bei Hunden Atemnotzustände, Koagulopathie und andere Symptome verursacht. Ziel dieser Studie war, die Seroprävalenz und Verbreitung von *A. vasorum* in Schweizer Hunden zu untersuchen sowie Korrelationen mit biogeographischen Aspekten zu identifizieren. Insgesamt 6136 Hundeseren wurden mittels ELISA auf spezifische Antikörper und zirkulierende Antigene untersucht. Die Seroprävalenz mit positivem Nachweis sowohl für Antikörper als auch für Antigen war 0.96%, während insgesamt 3.08% der Seren Antikörper- und 2.17% Antigen-positiv waren. Die höchste Prävalenz von gleichzeitig positivem Antikörper- und Antigennachweis wurde in der Westschweiz (Region Genf, 2.21%) ermittelt, welche ein neues endemisches Areal darstellt. Bekannte endemische Gebiete (Tessin 2.17%, Hochrheingebiet 1.11%) wurden bestätigt. Mittels Raumanalyse wurde ein Cluster von Antikörper-positiven Hunden in Sisseln (Kt. AG) ermittelt. Insgesamt 96.6% (57/59) der Antigen- und Antikörper-positiven Seren stammten aus Regionen mit durchschnittlicher Januar-Temperatur von mehr als -2 °C. Entsprechend kamen 89.8% (53/59) aus Regionen unter 700 m.ü.M. Diese Studie bestätigt bekannte endemische Gebiete und identifizierte positive Hunde in praktisch allen Bioregionen. Da asymptomatische Hunde eine plötzliche, potentiell tödliche Angiostrongylose entwickeln können, ist Wachsamkeit für Hunde aus Gebieten unter 700 m.ü.M. angezeigt.

Angiostrongylus vasorum – Hund – Antigennachweis – Antikörperrnachweis – Raumanalyse



Research paper

Seroepidemiological survey and spatial analysis of the occurrence of *Angiostrongylus vasorum* in Swiss dogs in relation to biogeographic aspects



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ABSTRACT

Angiostrongylus vasorum is a metastrongyloid nematode living in the pulmonary arteries and in the right heart causing potentially fatal respiratory distress, coagulopathies and a wide range of other clinical signs in dogs. The aim of this study was to investigate the seroprevalence and distribution of *A. vasorum* in Swiss dogs and to identify correlations with biogeographic aspects. A total of 6136 dog sera from all over the country submitted by veterinarians for haematological or chemical analyses were examined for the presence of circulating *A. vasorum* parasite antigen and specific antibodies against *A. vasorum* by ELISA. The combined seroprevalence for both specific antibodies and antigen was 0.96% (95% confidence intervals: 0.7–1.2%), while the overall antibody prevalence was 3.08% (CI: 2.7–3.5%) and the antigen prevalence 2.17% (CI: 1.8–2.6%). The highest prevalence for dogs identified as positive in both ELISAs was detected in Western Switzerland (around Geneva, 2.21%, CI: 0.7–5.1%), representing a new endemic area. Known endemic regions in Southern Switzerland (Ticino, 2.17%, CI: 1.0–4.0%) and in the High Rhine area (northern Switzerland, 1.11%, CI: 0.4–2.4%) were confirmed. Spatial analysis identified a cluster with a radius of approximately 30 km in Sisseln, located in this latter region at the German border, for antibody positive dogs, which interestingly corresponded to the location of historical cases of canine angiostrongylosis diagnosed 12–16 years previously. In total 96.6% (57/59) of the antigen- and antibody positive samples originated from areas with a mean temperature warmer than -2°C in January. Correspondingly, most of the samples (53/59, 89.8%) positive in both ELISAs originated from areas below 700 meters above sea level (m asl), suggesting the altitude being a limiting factor for *A. vasorum* transmission in Switzerland. This study confirms previously known endemic areas for *A. vasorum* in Switzerland, and additionally identifies positive dogs in virtually all bioregions. As asymptomatic dogs may suddenly develop a critical clinical status with potentially fatal outcome, disease awareness has to be maintained for dogs from across Switzerland at altitudes below 700 m asl.

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1. Introduction

Angiostrongylus vasorum is a metastrongylid nematode whose first description dates back to 1854 (Serres, 1854) when the parasite was detected in a dog in France. Dogs, foxes and other canids (Poli et al., 1984; Segovia et al., 2004; Bourque et al., 2005; Duarte et al., 2007) are definitive hosts which may become infected through the ingestion of intermediate hosts, i.e. slugs and snails containing the infective third-stage larvae (L3), or, possibly, directly with L3 surviving outside of the intermediate host (Ferdushy and Hasan, 2010). Starting from the tenth day after ingestion, the immature *A.*

vasorum stages settle in the right side of the heart and in the pulmonary arteries of the definitive host. It is only after a prepatency of 38–55 days that hatched first-stage larvae (L1) can be detected in the faeces after emerging from adult stages (Guilhon and Cens, 1973; Schnyder et al., 2009).

An infection with *A. vasorum* in dogs mainly affects the cardiorespiratory and coagulation system; however, the nervous system (Staebler et al., 2005; Negrin et al., 2008), the eyes (Rosenlund et al., 1991) or other organs may also be involved. The most frequent clinical signs are respiratory distress (coughing, dyspnoea, tachypnoea, exercise intolerance) and bleeding disorders (i.e. haemorrhage, mucosal bleeding, bleeding after surgery), but asymptomatic dogs or dogs with numerous other signs such as depression, anorexia, weight loss, vomiting, diarrhoea and neurological signs are frequently observed (reviewed in Koch and

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Willesen, 2009). The diagnosis can be challenging because of the variable and non-pathognomonic clinical manifestations and also because the infection can remain unnoticed for months or even years. Due to the often chronic course of the infection, clinical signs may only become obvious to the owner when severe pathological changes have already occurred, with potential consequently fatal outcomes (Staebler et al., 2005; Chapman et al., 2004; Denk et al., 2009).

Recorded variations in the prevalence of *A. vasorum* in dogs depend on the diagnostic methods used and the study population, and are complicated by the fact that the geographic distribution of this parasite is not homogeneous: *A. vasorum* is known to be confined to endemic foci (Eckert and Lämmler, 1972; Martin and Neal, 1992), which may vary over time (Morgan, 2014). Prevalences of *A. vasorum* between 0.52% (Barutzki and Schaper, 2011) and 15.70% (Morgan et al., 2010) determined by faecal examination are described in dogs in different European countries. Endemic areas were first described in dogs and/or foxes from southern France (Cuillé and Darraspen, 1930; Guilhon, 1963; Bourdeau, 1993), Ireland (Dodd, 1973; Williams et al., 1985), south-east England and Wales (Jacobs and Prole, 1975; Simpson and Neal, 1982), Italy (Poli et al., 1984) and Denmark (Bolt et al., 1992). In Switzerland, *A. vasorum* was described first in a single breeding kennel in Zürich (Wolff et al., 1969), and only decades later in the region of Basel neighbouring Germany (both in the north of the country) and also in Ticino, south of the Alps neighbouring Italy (Staebler et al., 2005). Since 2009, an apparently increasing number of clinical cases have been diagnosed (M. Schnyder, personal communication). In the last decades the number of reports of angiostrongylosis has increased throughout Europe, indicating that the parasite is widely present, also outside of the previously known endemic areas. It is generally accepted that foxes, in which the prevalence of *A. vasorum* detected at necropsy varies between 5% and 80% in Europe (Sreter et al., 2003; Saeed et al., 2006; Magi et al., 2009a) and up to 56% on Newfoundland island (Jeffery et al., 2004), are definitive host representing an important wild reservoir.

In the past the most frequently used tool to diagnose an *A. vasorum* infection in dogs was the Baermann technique, a coproscopic method based on larval migration (Deplazes et al., 2013), followed by microscopic identification of the larvae. However, the following limitations have to be considered: reduction of larval vitality caused by transportation or storage of faecal samples, lack of detection during prepatency, intermittent larval excretion as well as challenging morphologic differentiation from other lungworms (Oliveira-Junior et al., 2006; Barutzki and Schaper, 2009; McGarry and Morgan, 2009; Schnyder et al., 2010b). Alternative coproscopic methods have been described, such as flotation, direct faecal smears (Humm and Adamantos, 2010), and broncho-alveolar lavages (Barçante et al., 2008), but these methods exhibit lower sensitivities. Molecular techniques (Jefferies et al., 2011) with high specificity were applied with blood, lung tissue, broncho-alveolar lavage fluid, endotracheal mucus, pharyngeal swabs and faecal samples, but are not in use for routine diagnosis or mass-screening. In addition, compared with recently developed serological tests (Schnyder et al., 2011; Schucan et al., 2012), ELISAs gave the earliest and most consistent results (Schnyder et al., 2015), representing a valid alternative to coproscopic analyses in particular for mass-screening in the context of epidemiological surveys, as previously shown (Guardone et al., 2013; Schnyder et al., 2013). Indeed, the ELISAs enable the rapid testing of a considerable number of sera with high sensitivity and specificity. While the ELISA detecting circulating antigen identifies dogs with an ongoing active infection, the ELISA for specific antibody detection identifies dogs which have had or actually have contact with the parasite. Particularly in areas where the distribution of the parasite is unknown or a very low prevalence is supposed, the combination of antigen- and antibody-

ELISA is more advisable: considering only samples positive for both ELISAs, the positive predictive value tends to one at low prevalence, and therefore the risk of false-positive results decreases (Schnyder et al., 2013).

The aim of this study was to investigate the seroprevalence of both circulating *A. vasorum* antigen and of specific antibodies in Swiss dogs in relation to biogeographic aspects of the country.

2. Materials and methods

Sera of 6136 dogs from all over Switzerland submitted by veterinarians for haematological or chemical analyses for different medical reasons were collected between 2010 and 2013, including corresponding data on the owner's postal code. Due to data protection, no further information concerning the animal or the animal owner was available. The sera were provided by 18 private veterinary clinics, from two Swiss private veterinary diagnostic laboratories (Labor Laupen AG, Bern; IDEXX and Diavet Labor AG, Bäch) and from the Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland. The serum samples were stored at -20°C and then analysed with ELISAs for the detection of circulating antigens of *A. vasorum* (Schnyder et al., 2011; sensitivity 95.7%, specificity 94.0%) and for the detection of specific antibodies against *A. vasorum* (Schucan et al., 2012; sensitivity 85.7%, specificity 98.8%). Each plate was run with two positive controls (sera from experimentally infected dogs), two negative controls (from uninfected laboratory dogs) and a conjugate control. A diluted positive serum was added twice on each plate to calculate a correction factor for adjustment between plates, as previously described (Schnyder et al., 2011).

Excel 2007 for Windows (Microsoft Corporation, Redmond USA) was used to calculate means, standard deviations and 95% confidential intervals (CI). The cut-off value was calculated as mean plus three standard deviations of 300 randomly selected sera. For the sera analysed with the antibody-ELISA using *A. vasorum* adult E/S antigen, the cut-off value was calculated as the mean plus four standard deviations.

Each sample was depicted on maps based on the Swiss postal code system. The program Quantum GIS 1.8.0 2012 (Quantum GIS Geographic Information System, Open Source Geospatial Foundation Project <http://qgis.osgeo.org>) was used to represent the geographic distribution of *A. vasorum*. Data about the biogeographic regions were obtained from the Federal Office for the Environment (FOEN, www.bafu.admin.ch): they were defined based on the patterns of distribution of flora and fauna, applying a purely statistical approach, which was adapted to communal boundaries (Gonseth et al., 2001). Data on mean temperatures and altitudes in Switzerland were obtained from the Swiss World Atlas (www.schweizerweltatlas.ch) and from the Federal Office of Topography swisstopo (www.toposhop.admin.ch), respectively.

Furthermore, a spatial analysis of the distribution of positive sera was carried out with the SaTScan™ software (Version 9.3.1, 2014, National Cancer Institute, Bethesda, MD, USA, (Kulldorff, 1997; Kulldorff and Information Management Services Inc., 2009)). The scan statistic test (purely spatial, Bernoulli) assessed disease distribution with the use of community centroids and a circular scan for cases (positive communities) and controls (negative communities). Maps were created with the geographical information system software Quantum GIS (Version 1.8.0 'Lisboa' 2012, www.qgis.org).

3. Results

The seroprevalences of *A. vasorum* in Swiss dogs are shown in Table 1. A total of 59 dogs (0.96%, CI: 0.7–1.2%) were seropositive

Table 1

Seroprevalence of *Angiostrongylus vasorum* in Swiss dogs within defined biogeographic regions. Dog sera ($n=6136$) supplied by 21 different sources were tested for the presence of circulating *A. vasorum* antigen and specific antibodies against *A. vasorum* with ELISAs performed as described in Schnyder et al. (2011) and Schucan et al. (2012).

Swiss biogeographic regions (number of tested samples)	Antigen- and antibody-positive (n, % and 95% CI)	Antigen-positive only (n, % and 95% CI)	Antibody-positive only (n, % and 95% CI)	Antigen-positive (n, % and 95% CI)	Antibody-positive (n, % and 95% CI)
1. Geneva (226)	5, 2.21 (0.7–5.1)	8, 3.54 (1.5–6.9)	7, 3.10 (1.3–6.3)	13, 5.75 (3.1–9.6)	12, 5.31 (2.8–9.1)
2. Southern Alps (461)	10, 2.17 (1.0–4.0)	4, 0.87 (0.2–2.2)	11, 2.39 (1.2–4.2)	14, 3.04 (1.7–5.0)	21, 4.56 (2.8–6.9)
3. Central Eastern Alps (62)	1, 1.61 (0.0–8.7)	0, 0.00 (0.0–4.7)	2, 3.23 (0.4–11.2)	1, 1.61 (0.0–8.7)	3, 4.84 (1.0–13.5)
4. High Rhine (543)	6, 1.11 (0.4–2.4)	11, 2.03 (1.0–3.6)	32, 5.89 (4.1–8.2)	17, 3.13 (1.8–5.0)	38, 7.00 (5.0–9.5)
5. Jura Mountains and Randen (539)	5, 0.93 (0.3–2.2)	7, 1.30 (0.5–2.7)	21, 3.90 (2.4–5.9)	12, 2.23 (1.2–3.9)	26, 4.82 (3.2–7.0)
6. Eastern Swiss Plateau (1917)	17, 0.89 (0.5–1.4)	16, 0.83 (0.5–1.4)	38, 1.98 (1.4–2.7)	33, 1.72 (1.2–2.4)	55, 2.87 (2.2–3.7)
7. Northern Alps (371)	3, 0.81 (0.2–2.3)	4, 1.08 (0.3–2.7)	0, 0.00 (0.0–0.8)	7, 1.89 (0.8–3.8)	3, 0.81 (0.2–2.3)
8. Western Swiss Plateau (1537)	10, 0.65 (0.3–1.2)	15, 0.98 (0.5–1.6)	17, 1.11 (0.6–1.8)	25, 1.63 (1.1–2.4)	27, 1.76 (1.2–2.5)
9. Swiss Prealps (319)	2, 0.63 (0.1–2.2)	6, 1.88 (0.7–4.0)	2, 0.63 (0.1–2.2)	8, 2.51 (1.1–4.9)	4, 1.25 (0.3–3.2)
10. Central Western Alps (161)	0, 0.00 (0.0–1.8)	3, 1.86 (0.4–5.3)	0, 0.00 (0.0–1.8)	3, 1.86 (0.4–5.3)	0, 0.00 (0.0–1.8)
Total (6131)	59, 0.96 (0.7–1.2)	74, 1.21 (0.9–1.5)	130, 2.12 (1.8–2.5)	133, 2.17 (1.8–2.6)	189, 3.08 (2.7–3.5)

CI: confidence intervals.

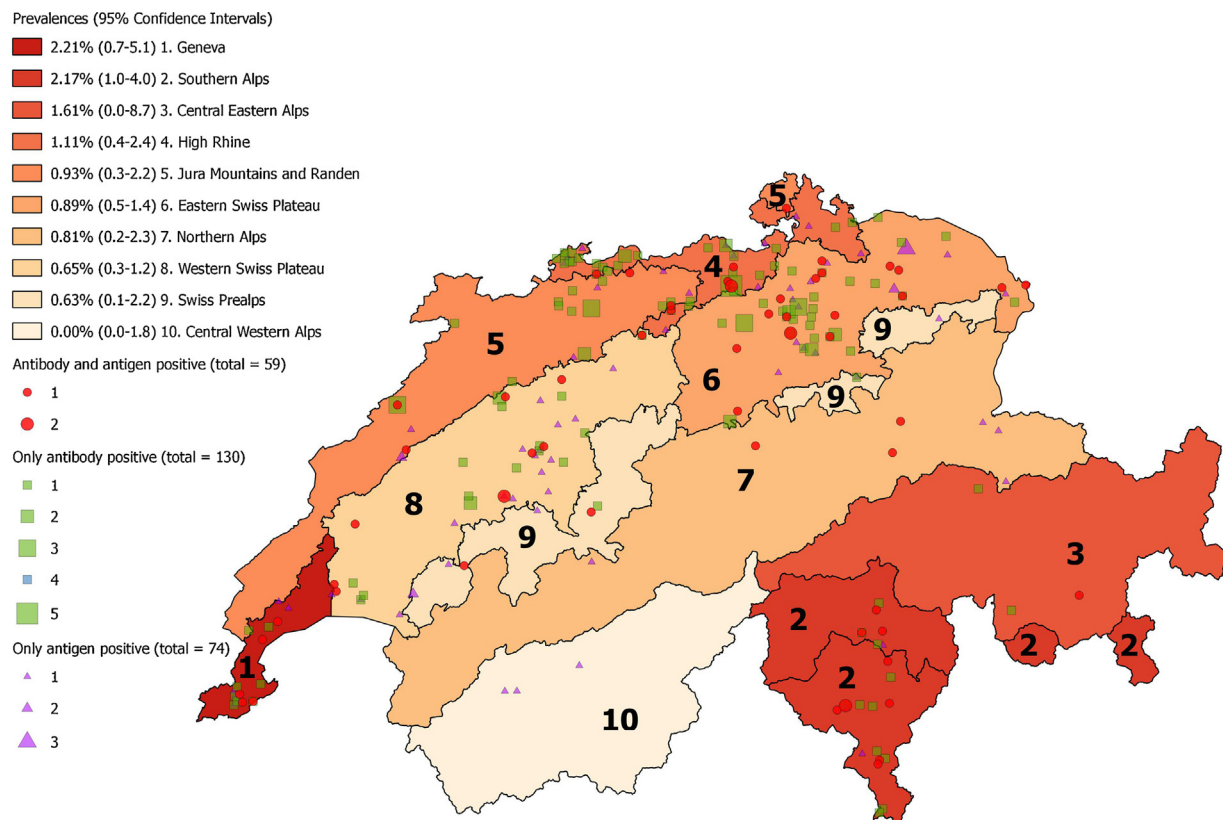


Fig. 1. Seroprevalences for *Angiostrongylus vasorum* infections in dogs within defined biogeographic areas of Switzerland. The 6136 dogs were tested for circulating antigens (Schnyder et al., 2011) and specific antibodies (Schucan et al., 2012) by ELISA. Location of sera originating from dogs positive in both ELISAs (red circles, $n=59$), sera with positive antigen ELISA only ($n=74$) and sera with positive antibody ELISA only ($n=130$) are shown based on postcode data of the owner's domicile.

in both ELISAs, while 130 dogs (2.12%, CI: 1.8–2.5%) were positive for antibody detection only and 74 dogs (1.21%, CI: 0.9–1.5%) for antigen detection only. Overall, 133 dogs (2.17%, CI: 1.8–2.6%) were antigen-positive and 189 of the animals (3.08%, CI: 2.7–3.5%) were antibody-positive. Fig. 1 shows the geographical distribution of the positive samples and prevalences for dogs seropositive in both ELISAs within biogeographic regions. Prevalences within biogeographic regions varied between 0 (Central Western Alps) and 2.21% (Geneva) (Table 1), but differences were not significant. By contrast, a spatial analysis scanning for clusters with high rates (Bernoulli model), revealed a significant cluster with a radius of approximately 30 km for antibody positive dogs (46/603 positive dogs, compared to the 19 positive dogs which would be expected

for a random distribution) in Sisseln (Fig. 2, coordinates centroid: 47.549, 7.983 in the World Geodetic System 1984), a village situated on the Rhine river at the border to southern Germany.

Fig. 3 shows the 59 samples positive in both ELISAs in relation to the altitude. The Swiss Alps cross Switzerland from west to east, with peaks over 4000 m asl (meters above sea level). The highest situated positive sample was identified from a dog from a municipality located at around 1700 m asl (municipality of St. Moritz, 1700–3160 m asl), though most ($n=53$, 89.8%) of the 59 positive samples originated from areas below 700 m asl. Furthermore, in total 96.6% (57/59) of these samples originated from areas where the mean temperature in January is higher than -2°C (Fig. 4).

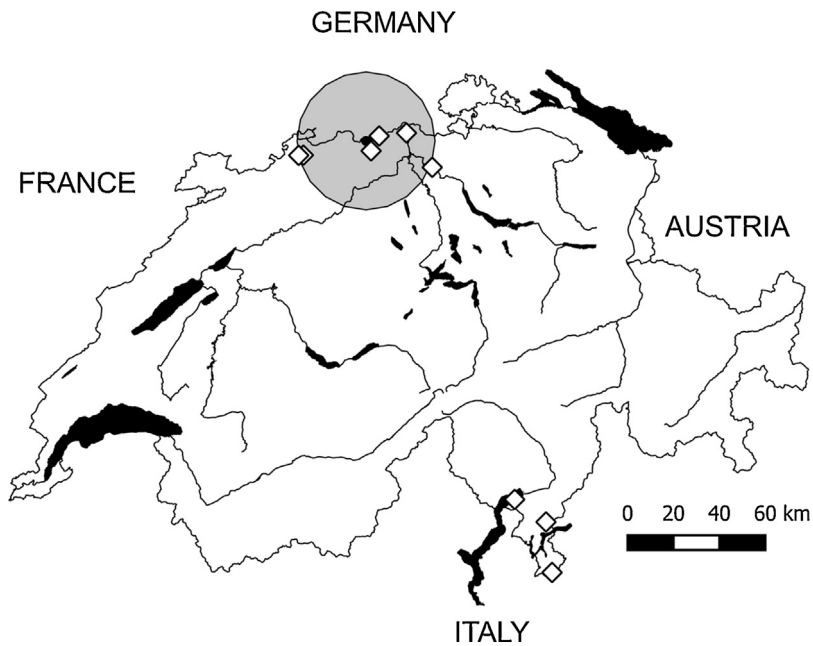


Fig. 2. Spatial analysis of the seroprevalence of Swiss dogs positive for specific antibodies against *Angiostrongylus vasorum* revealed a significant cluster in Sisseln (coordinates centroid: 47.549, 7.983 in the World Geodetic System 1984), close to the German border and including the location of six out of nine historical cases (◊) (Staeble et al., 2005).

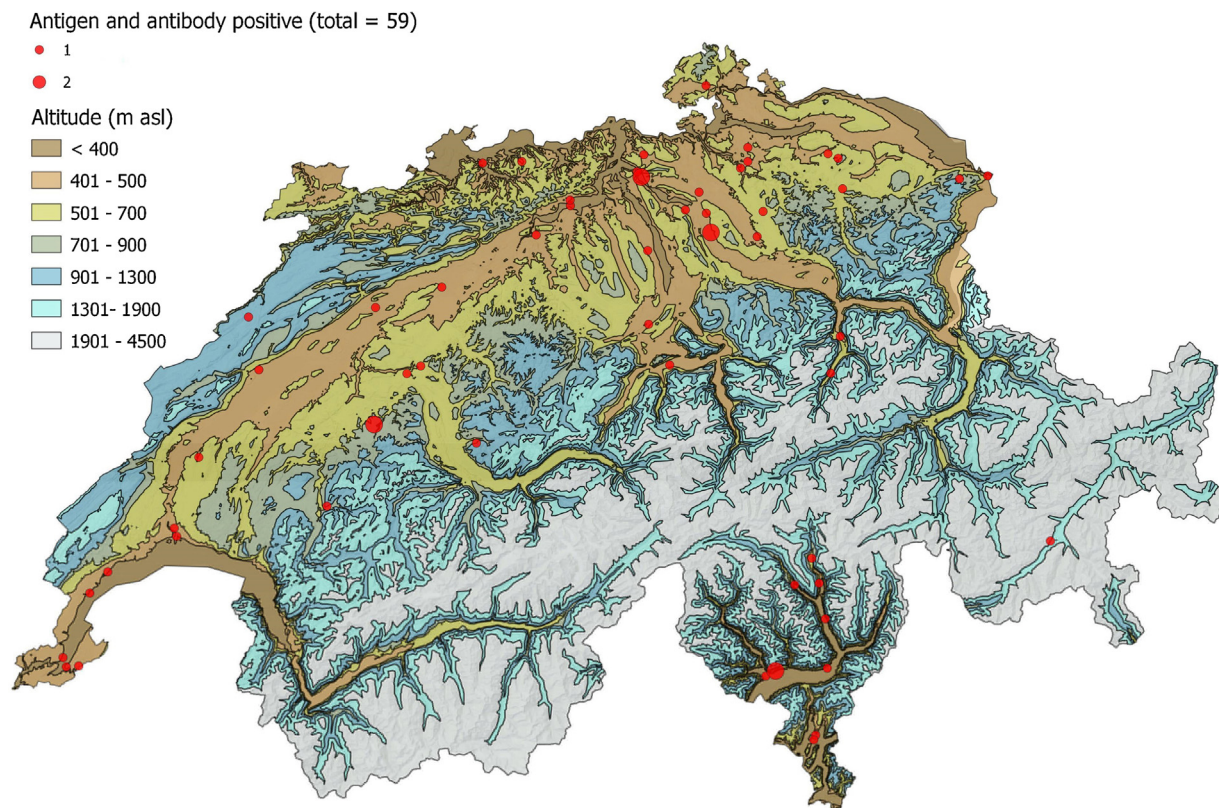


Fig. 3. Geographic location of canine sera with presence of both circulating antigen and specific antibodies against *Angiostrongylus vasorum*, in relation to altitude in meters above the sea level (m asl).

4. Discussion

The overall seroprevalence of 0.96%, 2.17% and 3.08% for dogs positive in both ELISAs, in antigen-ELISA and in antibody-ELISA respectively, confirms that Switzerland belongs to the countries in which *A. vasorum* is endemic. Antibody- and antigen-positive dogs

were distributed over large areas of the country where samples were collected. Comparing the seroprevalences of the 10 biogeographic regions of Switzerland, no statistically significant regional differences were observed, indicating that within Switzerland the patterns of distribution of flora and fauna are not relevant for the occurrence of *A. vasorum*. A possible trend for higher preva-

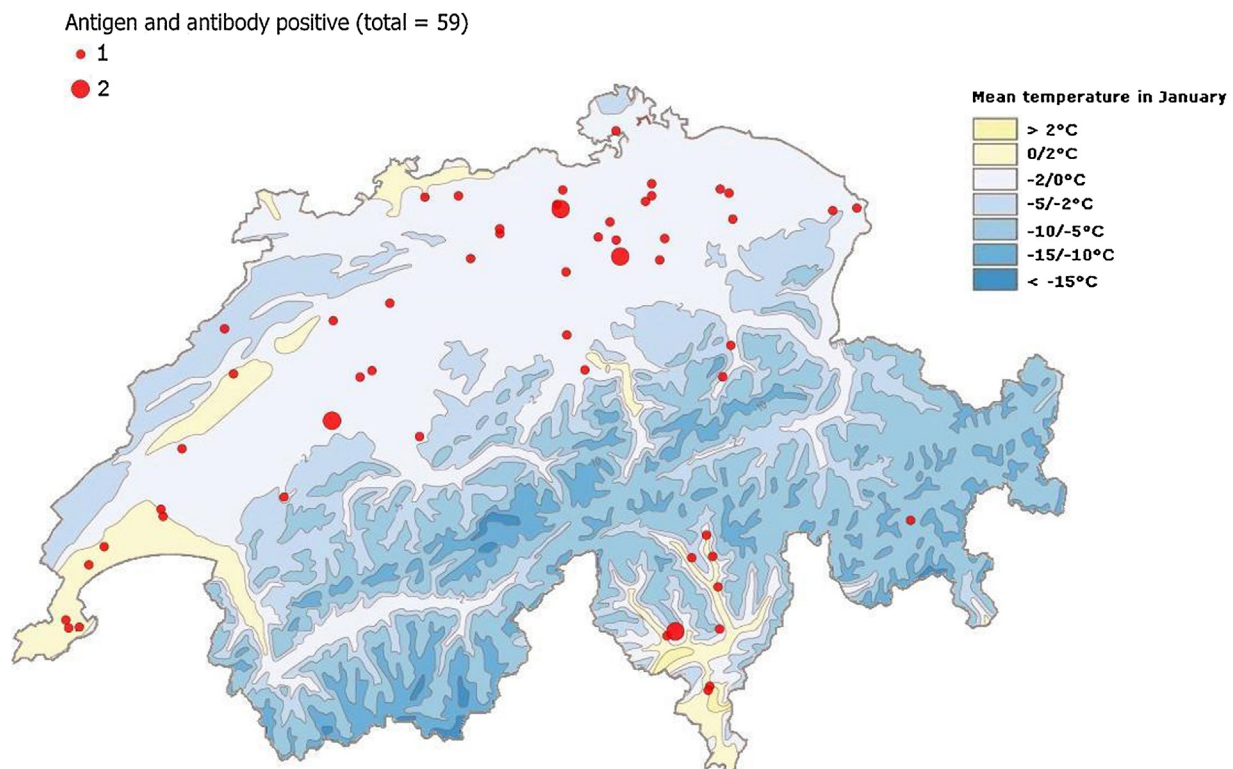


Fig. 4. Geographic location of canine sera with presence of both circulating antigen and specific antibodies against *Angiostrongylus vasorum*, in relation to the mean temperature in the month of January.

lence in the southern region of Ticino (Southern Alps) and in the region of Basel in the north (High Rhine) confirms that these areas represent classical endemic foci where *A. vasorum* was initially present and seems to persist: the previously described nine cases of canine angiostrongylosis diagnosed between 1999 and 2004 in Switzerland were originating from the same northern (six dogs) and southern (three dogs) areas (Staeble et al., 2005). This is interestingly confirmed by the spatial analysis performed in this study identifying the only significant cluster of antibody-positive dogs in the northern area of Switzerland bordering Germany, including the six just mentioned cases, which were identified between 1999 and 2003. In particular, the centre of the cluster is very close to the location of two of these cases. Therefore, this study also shows that a broadly performed seroepidemiological survey including spatial analysis of the results can help to identify endemic spots where a parasite first emerged in the past. Alternatively, a cluster of positive cases also could indicate spots where the circumstances (climate, interactions between final and intermediate hosts inter alia) are particularly favourable for transmission and establishment of *A. vasorum*. In this latter case, a continuous increase of prevalence is expected.

The notable prevalence (1.61%) in the Central eastern Alps may be explained by the restricted number of analysed sera ($n=61$) and the correspondingly wide confidence intervals (0.0–8.7%). By contrast, although not significant, the western region of Switzerland (around Geneva, with a prevalence of 2.21%) and the Southern Alps (with a prevalence of 2.17%) were the two areas with highest prevalence, and they intriguingly correspond to areas which were previously identified as having high suitability for parasite establishment (Morgan et al., 2009). These areas were identified based on a climate model approach supported by the comparison of climatic factors in areas with high parasitic presence and, at that stage, parasitic free areas. Whereas in southern Switzerland *A. vasorum* was diagnosed in dogs more than 10 years ago (Staeble et al.,

2005), the western region represents a newly identified important endemic area. A conspicuous concentration of dogs (Pospischil et al., 2013) and, more importantly, foxes (Deplazes et al., 2004; Reperant et al., 2007), could explain the high prevalence of *A. vasorum* infections in this urban area around the city of Geneva. In fact, previous studies showed that the prevalence of *A. vasorum* in the fox population was regularly higher than the one in dogs. For example, in areas northern and central Italy between 0.76 and 0.90% of the dogs were seropositive (Guardone et al., 2013), while between 7% (Magi et al., 2009a, b) and 39% (Poli et al., 1991) of the foxes were infected with *A. vasorum* at necropsy. Similarly, in England 0.97% (Schnyder et al., 2013) of dogs from the south-east were seropositive, while 23% (Morgan et al., 2008) of the foxes from the same area were infected with *A. vasorum* at necropsy. By analogy with studies of *Echinococcus multilocularis*, the fox tapeworm, the massive increase of fox populations due to the successful combat against rabies has been identified to be responsible for an increased number of human alveolar echinococcosis cases (Schweiger et al., 2007). The increasing fox population especially in central urban areas and in the urban periphery overlaps with the areas where dogs have free-roaming possibilities (Deplazes et al., 2004). This could have contributed to the establishment of *A. vasorum* in such areas and to a higher infection pressure for dogs, which are frequently walked in the urban periphery and in recreational areas (Deplazes et al., 2004). Accordingly, the observation of foxes in the home garden of *A. vasorum* infected dogs is commonly reported by owners (Chapman et al., 2004; Gallagher et al., 2012). Therefore, in overlapping areas with contemporaneous presence of dogs and foxes, the role of foxes is doubtless important for transmission and establishment of parasites shared between dog and fox populations. A highly concentrated fox population seems to enable the persistence of *A. vasorum* in so-called endemic foci, where foxes may act as the main reservoir with dogs acting as sentinel animals (Eckert and Lämmler, 1972). By contrast, studies performed on the

development of infectious stages of *A. vasorum* at different temperatures in intermediate hosts indicate that the role of overwintered snails (and therefore for long-term establishment) is epidemiologically negligible (Morgan et al., 2014). In spite of this, it would be interesting to have additional information about the presence of *A. vasorum* in foxes and snails from the same regions, which would sustain the autochthonous establishment of the parasite.

Certainly, dogs may have acquired *A. vasorum* infections outside of their usual living space. Virtually no samples were available from high alpine areas, where the human (and therefore dog) population density is low. Nonetheless it is noteworthy that, while results show that virtually all Swiss biogeographic regions were essentially suitable for *A. vasorum*, most of the positive samples were originating from regions lower than 700 m asl, suggesting the altitude being a limiting factor for *A. vasorum* occurrence. In addition, the maps showing the *A. vasorum* distribution patterns in relation to the mean temperature in January and in relation to altitude were strongly correlated. Temperature and in minor measure also humidity have been considered important for the intermediate host population and their activity, and thus for the development of infectious L3 (Morgan et al., 2009). In Switzerland, snails such as *Euconulus fulvus* and *Arianta arbustorum* and slugs such as *Dero-ceras agreste* and *Arion fuscus* have been found at altitudes above 2000 m asl (Boschi, 2011). However, although under experimental conditions a great number of snails and slugs showed suitability for successful development of infectious stages (reviewed in Eckert and Lämmler, 1972), the number of gastropod species infected with *A. vasorum* larvae in field surveys is restricted. Primarily slug species were naturally infected, especially *Arion* spp. (*rufus*, *lusitanus*, *ater*, *distinctus*), *Limax maximus* and *Tandonia sowerbyi* (Guilhon and Bressou, 1960; Eckert and Lämmler, 1972; Ferdushy et al., 2009; Patel et al., 2014), and recently also the snail *Helix aspersa* was found PCR-positive for *A. vasorum* (Helm et al., 2015). The occurrence of these species is acknowledged also for Switzerland (with the exception of *T. sowerbyi*), and their natural habitat is mostly situated at altitudes from 200 to 1100 m asl, though they can also be found at altitudes as high as 2000 m asl (Boschi, 2011). Recent surveys performed on snails and slugs ($n=266$, including 48 snails recognised to act as potential naturally infected intermediate hosts) from known endemic areas of Switzerland did not succeed in detecting *A. vasorum*. These results (prevalence: 0%, 95% CI: 0–1.1%) indicate that the transmission pathways in Switzerland are not fully clear and that a significant number of gastropods needs to be analysed in order to identify the intermediate hosts (Häni, 2015). Also, further and yet unknown factors involved in transmission and establishment of *A. vasorum* are supposed to cause differences of suitability, even in superficially similar areas (Morgan, 2014; Schnyder, 2015). Respective to the population of L1, it was experimentally shown that these stages survive at 5–16 °C (Ferdushy and Hasan, 2010). Based on necropsies of foxes in a surveillance study in Newfoundland, the northern limit of distribution of *A. vasorum* corresponded closely to the average winter 0 to –4 °C thermocline (December–February) (Jeffery et al., 2004). In our study, 97% of the positive samples in both ELISAs were located in areas with a mean temperature warmer than –2 °C in January. These results again are indicative of the important role of temperature and, correspondingly, of the altitude for the occurrence of *A. vasorum* in Switzerland.

The prevalences detected in this study are, applying the same test combination, comparable to those from southern England (0.97%, 1.32% and 3.20% for dogs positive in both ELISAs, in antigen-ELISA and in antibody-ELISA, respectively) and from Italy (0.76–0.90%, 1.41% and 2.67%, respectively), both countries where *A. vasorum* has been endemic for several decades (Jacobs and Prole, 1975; Poli et al., 1984). All studies, including those presented here, were performed with samples that were submitted by veterinarians

for miscellaneous but unknown medical reasons, including cardiorespiratory signs. This may represent a limitation concerning the representativeness of the tested samples in view of the whole dog population; however, considering that *A. vasorum* infections can remain unnoticed for a long time, the dataset could be considered to be representative of an average canine population, and the spatial patterns identified in this study are not affected by the sampling method. The implications of positive results with each (specific antibody and antigen detection) or both individual tests have been previously discussed (Schnyder et al., 2013). A combined positive result is indicative of a dog harbouring an active *A. vasorum* infection inducing an immunological reaction with production of antibodies, and also has higher specificity than that of either individual test, although sensitivity is lower. This approach has been used to investigate the influence of temperature and altitude in this study, because areas with unknown prevalence have been sampled. Since the positive predictive value depends on the prevalence of the infection within the tested population, the contemporaneous use of the two tests needs to be considered in view of the test purposes (epidemiological survey in areas with low expected prevalence vs. early and sensitive individual diagnosis) and the sample size (Schnyder et al., 2013). The reasons for dogs seropositive for antigen or antibody detection only may depend on the different chronological detection by the two ELISAs at early stages of infections or after anthelmintic treatment, or on unknown individual differences among dogs and their immunological reactions, or on test inherent sensitivity and specificity.

In conclusion, we have confirmed known and identified new endemic areas for the presence of *A. vasorum* in dogs in Switzerland. In these areas veterinary practitioners could recommend a prophylactic monthly deworming or regular *A. vasorum* testing for the prompt diagnosis of positive dogs, as previously suggested (Koch and Willeßen, 2009; Schnyder et al., 2010a, b). Indeed, asymptomatic and therefore unnoticed dogs may suddenly develop a critical clinical status with fatal outcome. A recently developed rapid device to be broadly applied in veterinary practices, in particular for dogs with suspected canine angiostrongylosis, is now commercially available and showed a good sensitivity and a very high specificity (Schnyder et al., 2014). In view of the distribution patterns identified herein, disease awareness has to be maintained also in low endemic and still not identified areas, especially at altitudes below 700 m asl with a mean temperature that does not sink below –2 °C during the coldest months of the year.

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